

# Genetic variation at *CR1* increases risk of cerebral amyloid angiopathy

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## ABSTRACT

**Objective:** Accumulated evidence suggests that a variant within the *CR1* gene (single nucleotide polymorphism rs6656401), known to increase risk for Alzheimer disease (AD), influences  $\beta$ -amyloid ( $A\beta$ ) deposition in brain tissue. Given the biologic overlap between AD and cerebral amyloid angiopathy (CAA), a leading cause of intracerebral hemorrhage (ICH) in elderly individuals, we investigated whether rs6656401 increases the risk of CAA-related ICH and influences vascular  $A\beta$  deposition.

**Methods:** We performed a case-control genetic association study of 89 individuals with CAA-related ICH and 280 individuals with ICH unrelated to CAA and compared them with 324 ICH-free control subjects. We also investigated the effect of rs6656401 on risk of recurrent CAA-ICH in a prospective longitudinal cohort of ICH survivors. Finally, association with severity of histopathologic CAA was investigated in 544 autopsy specimens from 2 longitudinal studies of aging.

**Results:** rs6656401 was associated with CAA-ICH (odds ratio [OR] = 1.61, 95% confidence interval [CI] 1.19–2.17,  $p = 8.0 \times 10^{-4}$ ) as well as with risk of recurrent CAA-ICH (hazard ratio = 1.35, 95% CI 1.04–1.76,  $p = 0.024$ ). Genotype at rs6656401 was also associated with severity of CAA pathology at autopsy (OR = 1.34, 95% CI 1.05–1.71,  $p = 0.009$ ). Adjustment for parenchymal amyloid burden did not cancel this effect, suggesting that, despite the correlation between parenchymal and vascular amyloid pathology, *CR1* acts independently on both processes, thus increasing risk of both AD and CAA.

**Conclusion:** The *CR1* variant rs6656401 influences risk and recurrence of CAA-ICH, as well as the severity of vascular amyloid deposition. *Neurology*® 2012;78:334–341

## GLOSSARY

**$A\beta$**  =  $\beta$ -amyloid; **AD** = Alzheimer disease; **BA** = Brodmann area; **CAA** = cerebral amyloid angiopathy; **CAA-ICH** = cerebral amyloid angiopathy-related intracerebral hemorrhage; **CI** = confidence interval; **GOCHA** = Genetics Of Cerebral Hemorrhage on Anticoagulation; **GWAS** = genome-wide association studies; **HR** = hazard ratio; **HTN-ICH** = hypertension-related intracerebral hemorrhage; **ICH** = intracerebral hemorrhage; **MAF** = minor allele frequency; **MAP** = Rush Memory and Aging Project; **OR** = odds ratio; **PCA** = principal component analysis; **ROS** = Religious Order Study; **SNP** = single nucleotide polymorphism.

Cerebral amyloid angiopathy (CAA) is characterized by  $\beta$ -amyloid ( $A\beta$ ) peptide deposition in the walls of arterial vessels of the cerebral cortex and cerebellum.<sup>1,2</sup> Like the amyloid plaques in Alzheimer disease (AD), vascular amyloid is composed of a proteolytic fragment ( $A\beta$ ) of the  $\beta$ -amyloid precursor protein.  $A\beta$  deposition is responsible for a variety of clinical consequences, including acute intracerebral hemorrhage (ICH).<sup>3–6</sup> CAA-related ICH<sup>7</sup> accounts for between 15% and 40% of all nontraumatic ICH in elderly individuals and is associated with mortality rates of 30%–50%.<sup>8–10</sup>

Supplemental data at  
[www.neurology.org](http://www.neurology.org)

Supplemental Data



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The biologic overlap between CAA and AD is substantial. CAA is found in up to 40% of individuals with a clinical diagnosis of AD who come to autopsy.<sup>11</sup> Furthermore, there appears to be substantial overlap in the genetics of the 2 conditions. Mutations in *APP* cause both autosomal-dominant AD and autosomal-dominant hereditary CAA-ICH.<sup>12,13</sup> In *APOE*, common polymorphisms influence risk of both sporadic CAA and sporadic AD.<sup>12,14</sup>

Genome-wide association studies (GWAS) have yielded a host of novel genetic risk factors for sporadic AD.<sup>15–17</sup> Of these, single nucleotide polymorphism (SNP) rs6656401 within the *CR1* gene has been associated with increased A $\beta$  deposition in brain specimens.<sup>18</sup> We therefore investigated whether this variant also influences risk of clinically symptomatic CAA-ICH, as well as histopathologic severity of CAA. We analyzed SNP data for CAA-ICH case and control subjects from the ongoing Genetics Of Cerebral Hemorrhage on Anticoagulation (GOCHA) study,<sup>14,19</sup> as well as for autopsied individuals enrolled in the Religious Order Study (ROS) and the Rush Memory and Aging Project (MAP).<sup>18,20–23</sup>

**METHODS** **GOCHA study. Subject recruitment.** Subjects were drawn from an ongoing multicenter genetic association study of primary and anticoagulation-related ICH.<sup>14,19</sup> In brief, study subjects are consecutive patients aged  $\geq 55$  years with ICH admitted to the emergency department of participating institutions (figure 1). Baseline head CT scans were reviewed centrally for determination of ICH location.

Race/ethnicity-, age-, and gender-matched control subjects were individuals enrolled from outpatient clinical services at participating institutions and were confirmed to have no medical history of ICH, AD, or pre-enrollment cognitive impairment through in-person interview and review of medical records.<sup>10,14,19</sup>

**Longitudinal follow-up.** All patients with ICH who survived at least 90 days after the index ICH were considered eligible for follow-up longitudinal analysis. Patients and their caregivers were interviewed by telephone at 3 months after ICH, 6 months after ICH, and every 6 months thereafter.<sup>10</sup> Information collected included medication use, recurrent lobar or nonlobar ICH, and death. If new neurologic symptoms, ischemic stroke, ICH, or hospital admission was reported by the subject or caregiver, the relevant medical records and radiographic images were reviewed by a study investigator blinded to other clinical and genetic data to assess the presence or absence of recurrent ICH. Events qualifying for censoring of subjects' data included clinically symptomatic ICH confirmed by neuroimaging, death, or follow-up period reaching the predetermined deadline for prospective ascertainment (January 1, 2009).

**Data collection and variable definition.** Clinical data were recorded at index ICH admission by stroke neurologists as part of routine clinical care. Collected data included information on demographics, previous medical history, Glasgow Coma Scale score, and pre-ICH medication use. All clinical and neuroimaging data were collected by individuals blinded to genotype data.

CAA-related ICH (CAA-ICH) was defined as lobar ICH (selective involvement of cerebral cortex or underlying white matter on admission CT scan) fulfilling the Boston criteria for a diagnosis of definite/probable CAA (i.e., demonstrating CAA pathology or multiple strictly lobar hemorrhagic lesions).<sup>7</sup>

To provide a negative control cohort for comparison purposes, we also analyzed data from deep hemispheric ICH (involving the basal ganglia, thalamus, or brainstem), typically caused by hypertensive vasculopathy (HTN-ICH). Subjects with cerebellar ICH and mixed location ICH were excluded from the analysis. Inclusion of these subjects ( $n = 9$ ) in either analysis group did not alter results (data not shown).

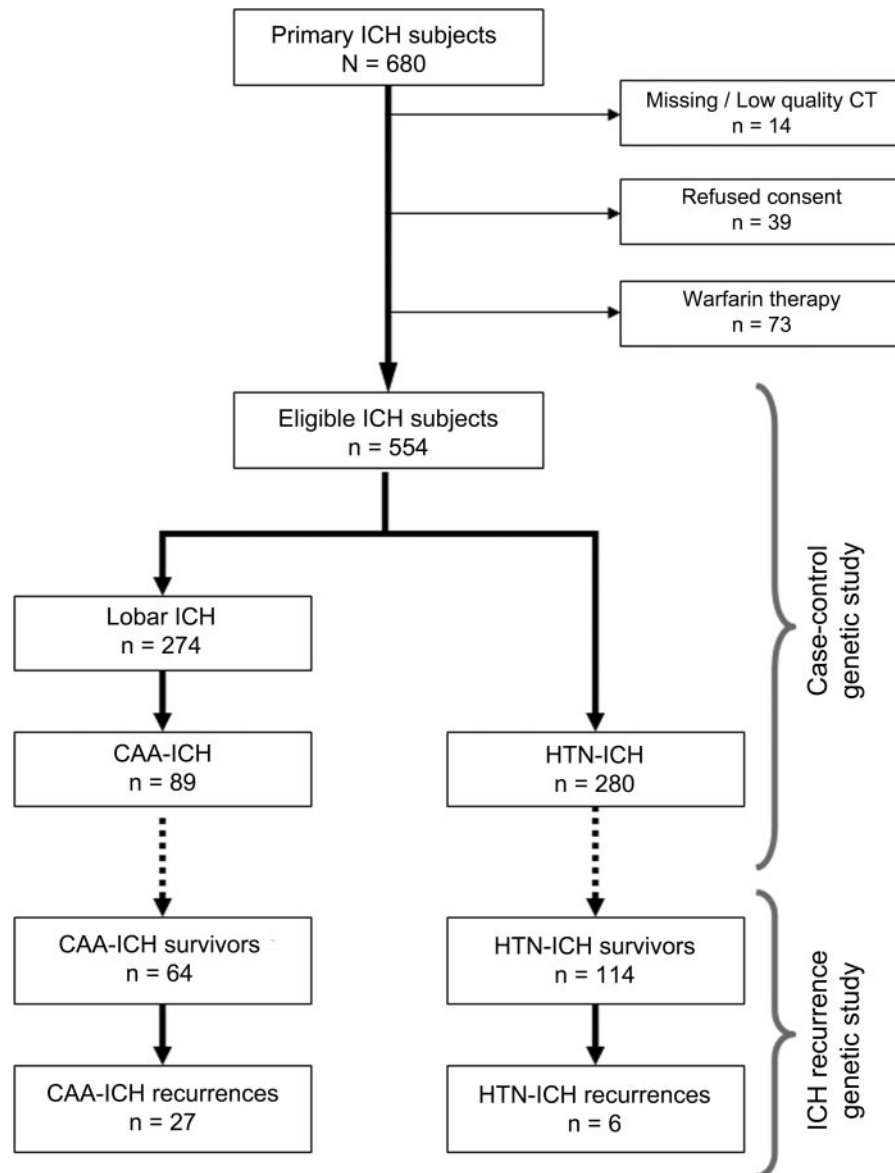
**ROS and MAP. Subject recruitment.** The ROS, started in 1994, enrolled older Catholic priests, nuns, and brothers, aged  $\geq 53$  years, from about 40 groups in 12 states.<sup>20</sup> Since January 1994, 1,132 participants, of whom 1,001 were non-Hispanic white, completed their baseline evaluation. The follow-up rate of survivors exceeds 90% as does the autopsy rate (481 autopsies of 511 deaths, of whom 457 were non-Hispanic white). Participants were free of known dementia at enrollment.

The MAP, started in 1997, enrolled older men and women (aged  $\geq 55$  years) free of known dementia from retirement communities in the Chicagoland area.<sup>21</sup> Since October 1997, 1,285 participants, of whom 1,118 were non-Hispanic white, completed their baseline evaluation. The follow-up rate of survivors exceeds 90%, and the autopsy rate exceeds 80% (336 autopsies of 411 deaths, of whom 320 were non-Hispanic white).

Participants in both studies agreed to annual clinical evaluations and signed both an informed consent and an Anatomic Gift Act form, donating their brains to Rush investigators at the time of death. All clinical and pathologic data were collected, and analyses were performed by study personnel blinded to genotype data. Likewise, cutoffs for CAA severity categorization were chosen according to previously published reports, without any knowledge of individuals' genetic data.<sup>20</sup> More detailed descriptions of these studies can be found in previously published literature.<sup>18,20–23</sup>

**Data collection and variable definition.** Brain autopsies were performed using standard techniques by investigators blinded to clinical data, as described previously.<sup>20,22</sup> In brief, CAA pathology was assessed by light microscopy on 20- $\mu$ m immunostained sections derived from 5 brain regions, including 4 neocortical regions—midfrontal (Brodmann area [BA] 46/9), inferior temporal (BA20), angular gyrus (BA39), and calcarine cortices (BA17)—and 1 mesial temporal region and the hip-

**Figure 1** Intracerebral hemorrhage (ICH) cohort sample size and study design



CAA-ICH = probable or definite cerebral amyloid angiopathy-related ICH; HTN-ICH = hypertension-related ICH in the deep brain structures.

pocampus. CAA was assessed in each region labeled with anti-A $\beta$  (clone 6F/3-dimensional, M0872, 1:100; DAKO) and quantified using a 5-point scale (0 through 4), with 0 = none (no immunohistostaining for CAA), 1 = mild (scattered positivity in either leptomeningeal or cortical blood vessels), 2 = moderate (strong, circumferential positivity in some but not all leptomeningeal or cortical blood vessels), 3 = severe (widespread, strong, circumferential positivity in leptomeningeal and cortical blood vessels), and 4 = very severe (same as 3, but with additional changes of positivity emanating from small cortical vessels into surrounding neuropil [dysphoric change]). All brains were also examined for pathologic markers of AD.

Because CAA severity was related across regions (all  $p < 0.0001$ ), we averaged the 5 regional scores to create an overall CAA severity score for each subject. Because the vast majority of subjects had some degree of CAA pathology (84.9%) and to make analyses easier to interpret, the overall CAA severity score

was converted into a 3-level class variable predictor. We used an overall severity score of  $>2.5$  to separate individuals with mild to moderate from those with moderate to very severe CAA, with the reference group consisting of persons with none-to-minimal CAA, defined by a score of  $<0.5$ . Separate, quantitative composite measures of neuritic and diffuse amyloid plaque pathology were calculated on the basis of counts from 5 brain regions (hippocampus, entorhinal cortex, midfrontal cortex, middle temporal cortex, and inferior parietal cortex) using the greatest density in a 1-mm<sup>2</sup> area of each region on modified Bielschowsky silver-stained 6- $\mu$ m sections, as described previously.<sup>24</sup> We standardized the raw counts by dividing each person's count by the SD for that particular count and formed summary scores by averaging the standardized scores.

**Genotype data.** All subjects included in the present study underwent GWAS SNP genotyping using the Illumina 610-Quad

array (GOCHA) or Affymetrix GeneChip 6.0 (ROS and MAP). Patients with ICH and control subjects were both genotyped in multiple batches, and each batch included both case and control subjects in an interspersed disposition on the genotyping plates. Batch assignment for ICH case and control subjects was determined randomly, irrespective of ICH phenotype (i.e., CAA-ICH vs non-CAA-ICH). Genotypes were called using BeadStudio software, and calls were confirmed manually for SNPs in the *CRI* gene region by laboratory personnel blinded to clinical phenotype. The ROS and MAP samples were genotyped on the Affymetrix 6.0 platform in a single run using identical protocols.

Both genome-wide datasets underwent stringent quality control procedures according to a previously published protocol using PLINK version 1.07.<sup>14,23,25</sup> In brief, quality control of genotype data included filters for missingness, heterozygosity, and concordance between genotype-determined and reported gender. SNP quality control included filters for minor allele frequency (MAF), missingness, Hardy-Weinberg equilibrium, and differential missingness by case-control status (figure e-1 on the *Neurology*<sup>®</sup> Web site at [www.neurology.org](http://www.neurology.org)). Individual genotypes for rs6656401 were extracted from GWAS data following quality control procedures and were found to be in Hardy-Weinberg equilibrium ( $p > 0.20$ ) in all 3 datasets (GOCHA, ROS, and MAP). Population structure was assessed by performing principal component analysis (PCA) on a subset of all SNPs selected using previously published criteria.<sup>14,26</sup> We assigned genotype-determined ancestry by comparing study subjects and reference populations from HapMap phase 3 data (figure e-2). To control for population stratification, all individuals analyzed

were confirmed to cluster with European HapMap samples to be eligible for analysis. Furthermore, principal components 1 and 2 (PC1 and PC2) from PCA analysis were used as covariates in all multivariate models (see below).<sup>14,25,26</sup>

**Statistical methods.** Genotype data were analyzed using an additive model, with odds ratio (OR) or hazard ratio (HR) expressing the effect of each copy of the reference allele. To determine the association between rs6656401 and CAA-ICH or HTN-ICH risk, we used logistic regression analyses. Results from different logistic regression models were compared using the Breslow-Day test. To determine the association between rs6656401 and CAA pathology, we used ordinal logistic regression analyses. We determined univariate predictors of ICH recurrence using Kaplan-Meier plots with significance testing by the log-rank test. For individuals with multiple recurrent CAA-ICH during follow-up, data were censored at the time of the first recurrence. To determine the influence of rs6656401 on CAA-ICH recurrence, we used Cox regression analysis. The proportional hazard assumption was tested using graphical checks and Schoenfeld residual-based tests.<sup>27</sup>

Covariates for all multivariate models included age, sex, PC1 and PC2, number of *APOE*  $\epsilon 2$  and  $\epsilon 4$  alleles, and history of hypertension. CAA pathology analyses were further adjusted for AD pathology to exclude confounding due to the known association with amyloid plaque burden.

Power calculations were performed using the Genetic Power Calculator ([pengu.mgh.harvard.edu/~purcell/gpc/](http://pengu.mgh.harvard.edu/~purcell/gpc/)).<sup>28</sup> Adjustment of effect size estimates due to the winner's curse was achieved using WINNER software ([csg.sph.umich.edu/boehnkewinner/](http://csg.sph.umich.edu/boehnkewinner/)).<sup>29</sup> This program modifies (reduces) the observed effect size estimate in genetic association studies based on available statistical power, as determined by sample size and  $\alpha$  threshold for significance.

All other association statistical analyses were performed using R software version 2.10.0 (The R Project for Statistical Computing, [www.r-project.org](http://www.r-project.org)). All significance tests were 2-tailed with significance threshold set at  $\alpha = 0.05$ .

**Standard protocol approvals, registrations, and patient consents.** The GOCHA study is conducted with approval of the institutional review boards of the Massachusetts General Hospital and all other enrolling institutions. The ROS and MAP studies were approved by the institutional review board of Rush University. All subjects enrolled in the present study (or their guardians) provided written informed consent before participation.

**RESULTS Characteristics of ICH case and control subjects.** A total of 554 subjects with ICH presented to GOCHA study centers from 2003 to 2009, were genotyped on a GWAS array, and passed all quality control filters (table 1). Of these, 89 individuals qualified for a diagnosis of CAA-ICH, and 280 individuals were classified as having HTN-ICH (table 1). A total of 324 ICH-free control subjects of European ancestry were available for analysis. In comparison with subjects with HTN-ICH, individuals with CAA-ICH were 1) older, 2) less likely to have a history of pre-ICH hypertension or diabetes, 3) more likely to have had a prior ICH before the index event, 4) more likely to have pre-ICH cognitive impair-

**Table 1** Characteristic of subjects included in ICH case-control analysis (GOCHA study)<sup>a</sup>

	CAA-ICH (n = 89) <sup>b</sup>	HTN-ICH (n = 280)	Control subjects (n = 324)
<b>Clinical variables</b>			
Age, y, mean $\pm$ SD	75.1 $\pm$ 9.4	70.6 $\pm$ 12.1	73.1 $\pm$ 8.0
Gender, n (% female)	44 (49.4)	126 (45.0)	147 (45.4)
History of hypertension	56 (62.9)	237 (84.6)	216 (66.6)
Ischemic heart disease	19 (21.3)	68 (24.3)	67 (20.7)
Atrial fibrillation	19 (21.3)	58 (20.7)	71 (21.9)
Type 2 diabetes	14 (15.7)	65 (23.2)	49 (15.1)
Prior functional dependence	3 (3.4)	5 (1.7)	0 (0.0)
Prior cognitive impairment	26 (29.2)	28 (10.0)	0 (0.0)
Pre-index ICH	12 (13.5)	10 (3.6)	0 (0.0)
<b>Pre-ICH medication use</b>			
Antiplatelet agents	32 (36.0)	109 (38.9)	136 (42.0)
Statins	26 (29.2)	73 (26.1)	80 (24.7)
<b>Genetic variables</b>			
rs6656401 (A allele, MAF)	0.24	0.19	0.18
<i>APOE</i> $\epsilon 2$ (MAF)	0.18	0.10	0.10
<i>APOE</i> $\epsilon 4$ (MAF)	0.24	0.14	0.12

Abbreviations: CAA-ICH = cerebral amyloid angiopathy-related intracerebral hemorrhage; GOCHA = Genetics Of Cerebral Hemorrhage on Anticoagulation; HTN-ICH = hypertension-related intracerebral hemorrhage; ICH = intracerebral hemorrhage; MAF = minor allele frequency.

<sup>a</sup> All variables are reported as sample size (percentage) unless otherwise specified.

<sup>b</sup> Defined as lobar ICH with diagnosis of definite/probable CAA according to the Boston criteria.<sup>1</sup>



**Table 2** Characteristics of prospectively followed ICH survivors (GOCHA study)<sup>a</sup>

	CAA-ICH (n = 64) <sup>b</sup>	Nonlobar ICH
<b>Clinical variables</b>		
Age, y, mean ± SD	74.4 ± 7.8	68.4 ± 13.0
Gender, n (% female)	28 (43.8)	47 (41.3)
History of hypertension	33 (51.6)	99 (86.8)
Ischemic heart disease	9 (14.0)	26 (22.8)
Atrial fibrillation	11 (17.2)	16 (14.0)
Type 2 diabetes	3 (4.7)	29 (25.4)
Prior functional dependence	2 (3.1)	2 (1.9)
Prior cognitive impairment	8 (12.5)	14 (12.3)
Pre-index ICH	8 (12.5)	5 (4.3)
<b>Pre-ICH medication use</b>		
Antiplatelet agents	11 (17.2)	14 (21.9)
Statins	4 (6.3)	29 (25.4)
<b>Genetic variables</b>		
rs6656401 (A allele, MAF)	0.24	0.18
APOE ε2 (MAF)	0.18	0.09
APOE ε4 (MAF)	0.25	0.15
<b>Longitudinal endpoints</b>		
ICH recurrence: CAA-ICH	27 (42.2)	0 (0.0)
ICH recurrence: HTN-ICH	0 (0.0)	6 (5.3)

Abbreviations: CAA-ICH = cerebral amyloid angiopathy-related intracerebral hemorrhage; GOCHA = Genetics Of Cerebral Hemorrhage on Anticoagulation; HTN-ICH = hypertension-related intracerebral hemorrhage; ICH = intracerebral hemorrhage; MAF = minor allele frequency. <sup>a</sup> All variables are reported as absolute number (percentage) unless otherwise specified.

<sup>b</sup> Subset of all lobar ICH qualifying for a diagnosis of probable CAA according to the Boston criteria.<sup>1</sup>

ment, and 5) more likely to possess both *APOE* ε2 and ε4 alleles (all  $p < 0.05$ ).

A total of 178 subjects with ICH (64 with CAA-ICH and 114 with HTN-ICH) were eligible for follow-up and ICH recurrence analysis (table 2). During a median follow-up time of 34.3 months (interquartile range 15.1–57.6 months), we observed 27 recurrent CAA-ICH events and 6 HTN-ICH events. Consistent with previous reports, hemorrhage recurrence was indeed more frequent in CAA-ICH survivors (cumulative 2-year recurrence rate of 15.7% vs 3.4%,  $p = 0.011$ ).<sup>10</sup>

**Risk of CAA-ICH.** The A allele of SNP rs6656401 was associated with risk of CAA-ICH (OR = 1.61, 95% confidence interval [CI] 1.19–2.17,  $p = 8.0 \times 10^{-4}$ ) after multivariate analysis adjustments. No association was found for HTN-ICH (OR = 0.93, 95% CI 0.82–1.05,  $p = 0.30$ ). Comparison of effect sizes confirmed that strength of association for

rs6656401 differed in CAA-ICH and HTN-ICH (Breslow-Day  $p = 0.01$ ).

A post hoc power calculation returned statistical power for discovery of association within the CAA-ICH subset of 41%. Based on this finding, adjustment for the winner's curse estimated an effect size for rs6656401 in CAA-ICH of OR = 1.35.

**Risk of recurrent CAA-ICH.** In multivariate Cox regression analyses, survivors of CAA-ICH who possessed the A allele of rs6656401 were at increased risk for recurrent CAA-ICH (HR = 1.35, 95% CI 1.04–1.76,  $p = 0.024$ ) (figure 2). We found no evidence of association between rs6656401 and ICH recurrence in HTN-ICH survivors (HR = 1.03, 95% CI 0.57–1.87,  $p = 0.91$ ).

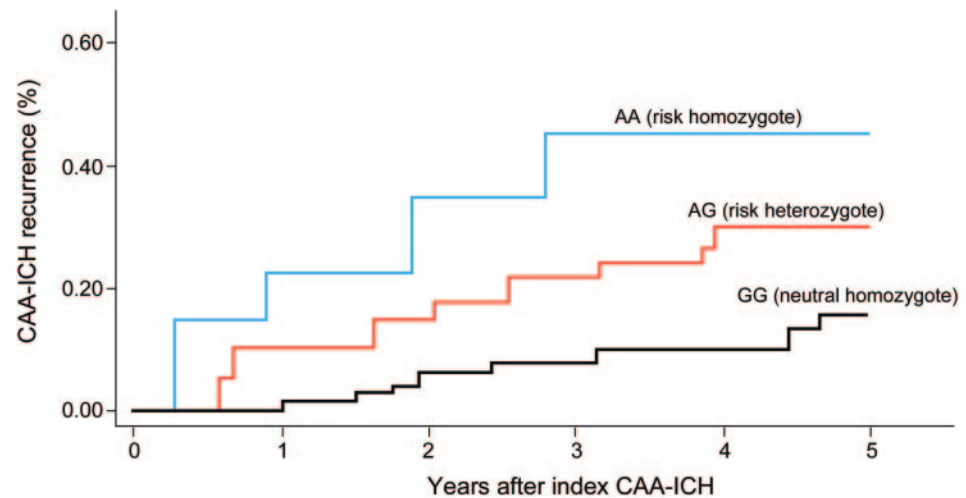
Our estimated power for discovery of this genetic association was 31%. Adjustment for the winner's curse returned an effect size of HR = 1.24.

**Histopathologic CAA in the ROS-MAP cohort.** A total of 544 subjects from the MAP and ROS cohorts had autopsy pathology data for CAA and AD as well as genome-wide data fulfilling quality control criteria (table 3). An increase in the number of copies of the A allele at rs6656401 was associated with increasing CAA pathology burden (none to minimal vs mild to moderate vs moderate to very severe)<sup>21–24</sup> in ordinal logistic regression (OR = 1.27, 95% CI 1.06–1.53,  $p = 0.009$ ). Adjustment for coexistent AD amyloid plaque burden (i.e., neuritic plaque and diffuse plaque pathology scores) did not significantly alter results (OR = 1.24,  $p = 0.01$ ).

**DISCUSSION** Results from our analyses demonstrate an association of the A allele of rs6656401 at the *CRI* locus with risk of CAA-ICH, risk of recurrent CAA-ICH, and CAA pathology burden. These findings mirror published reports linking *CRI* with parenchymal amyloid deposition in pathology samples and provide evidence of a possible biologic connection between CAA and AD.

Our study has limited statistical power (because of the limited sample size) to identify associations between rs6656401 and CAA-related phenotypes, particularly if the effect sizes typically observed for common genetic variants in common diseases are expected. The comparatively large increases in risk of CAA-ICH incidence/recurrence conferred by the rs6656401 genotype observed in the present study are therefore likely to be partially explained by the phenomenon of the winner's curse, i.e., systematic effect size overestimation by small (and therefore underpowered) studies.<sup>29,30</sup> Future replication and extension studies of the role of *CRI* in CAA and CAA-ICH should therefore be powered to detect

**Figure 2** Kaplan-Meier plot for recurrent cerebral amyloid angiopathy-related intracerebral hemorrhage (CAA-ICH) in CAA-ICH survivors based on rs6656401 genotype status



Different lines represent different individual genotypes at rs6656401. Failure rate estimates are adjusted for age, gender, pre-enrollment ICH, warfarin use, aspirin use, number of APOE  $\epsilon$ 2 and  $\epsilon$ 4 alleles, and principal components 1 and 2.

smaller effect sizes than those reported in the present study.

Of note, SNP rs6656401 shows significant stratification when different ethnic groups are compared; HapMap phase 3 data reports MAF >20% for European populations, MAF ~10% for Mexican Americans, and MAF <5% for Han Chinese, Chinese American, Japanese, Indian Gujarati, and African (Kenya) reference samples ([www.hapmap.org](http://www.hapmap.org)). Future studies will therefore also require application of tools for control of bias due to genetic ancestry, and power calculations will have to be tailored to specific allele frequencies in the populations being investigated.

Winner's curse adjusted effect size estimates for SNP rs6656401 in CAA are still larger than reported association results in recently published AD genome-wide studies.<sup>15–17</sup> One potential explanation is that *CRI* genetic variation acts on AD and CAA through different pathologic mechanisms, in whole or in part. A more likely explanation is that the phenotypic CAA classification, provided by neuroimaging (using the Boston criteria) or pathologic data, has greater accuracy than the AD diagnosis, which relies solely on clinical data in many studies.<sup>31</sup> Indeed, a recently published case-control association study of neuropathology-confirmed cases of AD re-

**Table 3** Characteristics of ROS and MAP subjects

	CAA Severity		
	None to minimal (n = 146)	Mild to moderate (n = 280)	Moderate to very severe (n = 118)
<b>Demographic variables</b>			
Age at enrollment, y, mean (SD)	84.4 (7.2)	86.3 (7.1)	88.6 (6.9)
Gender, n (% female)	80 (55.0)	160 (57.0)	73 (62.0)
Pre-enrollment history of dementia, n (%)	33 (23.0)	122 (44.0)	74 (63.0)
<b>Alzheimer disease pathology</b>			
Neuritic plaques pathology score median (IQR) <sup>a</sup>	0.0 (0.0–0.00)	0.87 (0.54–1.11)	1.10 (0.88–1.31)
Diffuse plaques pathology score, median (IQR) <sup>a</sup>	0.0 (0.0–0.23)	0.84 (0.51–1.17)	1.02 (0.78–1.30)
<b>Genetic variables</b>			
rs6656401 (A allele, MAF)	0.18	0.21	0.23
APOE $\epsilon$ 2 (MAF)	0.13	0.07	0.04
APOE $\epsilon$ 4 (MAF)	0.06	0.15	0.27

Abbreviations: CAA = cerebral amyloid angiopathy; IQR = interquartile range; MAF = minor allele frequency; MAP = Rush Memory and Aging Project; ROS = Religious Order Study.

<sup>a</sup> Calculated for each pathology feature by dividing each person's count by the SD for that particular count to standardize individual subjects' values.

ported larger effect sizes than those observed in discovery analyses. These pathology-informed AD studies uncovered effect sizes for SNP rs6656401 in the range we observed for CAA, rather than the smaller estimates generated in studies of clinically diagnosed AD.<sup>32</sup>

The association between rs6656401 and CAA-ICH risk and recurrence is potentially confounded by the genetic effects this variant exerts on parenchymal amyloid burden, which in turn correlates with CAA severity.<sup>18,20</sup> However, we found a significant association between rs6656401 and increasing severity of vascular amyloid deposition in autopsy samples from initially healthy community-dwelling elderly subjects. Adjustment for AD pathology in these analyses did not cancel the observed effect, suggesting that, despite the correlation between parenchymal and vascular amyloid burden,<sup>20</sup> *CRI* probably acts independently on both processes, thus increasing the risk of both AD and CAA. This finding echoes evidence from genetic association studies of *APOE*, in which the  $\epsilon 2$  allele decreases AD risk but increases risk of CAA.<sup>12,14</sup> Taken together, evidence from genetic association studies is consistent with multiple experimental observations in suggesting that, although etiologically related, AD and CAA represent different pathologic and clinical conditions.

Our study is limited by the challenges of assembling adequate sample sizes in diverse populations for a genetic study of CAA. We present 3 independent analyses that provide biologically consistent results, but we are not able to provide independent replication at this time. Adequate samples for replication are limited, although ongoing efforts in the international community are likely to generate such samples in the future. None of the reported associations achieve genome-wide significance ( $p < 0.5 \times 10^{-8}$ ), which would improve the robustness of our findings. However, a large body of preexisting data in AD genetics suggests that our findings are consistent with the known or suspected biologic functions of the *CRI* gene product. Finally, because of the restrictions imposed by genotyping procedures and study design, we are not able to extend our findings to non-European ancestry populations at this time. Additional studies will be required to clarify the role of *CRI* in these populations.

## AUTHOR CONTRIBUTIONS

Study concept: Dr. Biffi, Dr. Rosand. Study design: Dr. Biffi, Dr. Rosand. Acquisition of data: Dr. Biffi, Dr. Shulman, Dr. Jagiella, L. Cortellini, A.M. Ayres, K. Schwab, Dr. Brown, Dr. Silliman, Dr. Selim, Dr. Worrall, Dr. Meschia, Dr. Slowik, Dr. De Jager, Dr. Greenberg, Dr. Schneider, Dr. Bennett, Dr. Rosand. Statistical analysis: Dr. Biffi. Manuscript preparation: Dr. Biffi, Dr. Rosand. Manuscript review: Dr. Biffi, Dr. Shulman, Dr. Jagiella, L. Cortellini, A.M. Ayres, K. Schwab, Dr. Brown, Dr. Silliman, Dr. Selim, Dr. Worrall, Dr. Meschia, Dr. Slowik,

Dr. De Jager, Dr. Greenberg, Dr. Schneider, Dr. Bennett, Dr. Rosand. Study supervision/coordination: Dr. Biffi, L. Cortellini, A.M. Ayres, K. Schwab, Dr. De Jager, Dr. Schneider, Dr. Bennett, Dr. Rosand. Study funding: Dr. De Jager, Dr. Greenberg, Dr. Schneider, Dr. Bennett, Dr. Rosand.

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## DISCLOSURE

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